Setomelanomma holmii (Pleosporales, Phaeosphaeriaceae) on living spruce twigs in Europe and North America

Amy Y. Rossman, David F. Farr, Lisa A. Castlebury, Robert Shoemaker, and Alemu Mengistu

Abstract: *Setomelanomma holmii* M. Morelet, previously known only from the type specimen in France, was discovered in the U.S.A. (Kansas and Wisconsin) and Canada (Ontario) on living twigs of spruce (*Picea pungens* and *Picea glauca*). This fungus was grown from ascospores and compared with the ex-holotype culture. Morphology and ITS rDNA sequence similarities indicate that *S. holmii* belongs in the Pleosporales, Phaeosphaeriaceae. Sequence analysis of the SSU nrDNA places *S. holmii* in a clade containing members of the Leptosphaeriaceae and Phaeosphaeriaceae. *Setomelanomma holmii* is redescribed and illustrated based on the holotype and North American specimens.

Key words: Loculoascomycetes, Phaeosphaeriaceae, *Picea*, Pleosporales, needle chlorosis.

Résumé : Le *Setomelanomma holmii* M. Morelet, jusqu'à présent connu seulement en France par le spécimen type, a été retrouvé aux États-Unis (Kansas et Wisconsin) et au Canada (Ontario) sur des rameaux vivants d'épicéa (*Picea pungens* et *Picea glauca*). Ce champignon a été cultivé à partir d'ascospores et comparé avec une culture produite à partir de l'holotype. Les similarités dans la morphologie et les séquences ITS rDNA indiquent que le *S. holmii* appartient aux Pléosporales, Phaeosphaeriaceae. L'analyse des séquences des SSU nrDNA place le *S. holmii* dans un clade contenant des membres des Leptosphaeriaceae et des Phaeosphaeriaceae. Le *Setomelanomma holmii* est redécrit et illustré sur la base de l'holotype et des échantillons nord-américains.

Mots clés : Loculoascomycetes, Phaeosphaeriaceae, *Picea*, Pléosporales, brûlure des rameaux.

Introduction

Setomelanomma holmii M. Morelet was observed on samples of Colorado blue spruce (*Picea pungens* Engelm.) from Wisconsin, U.S.A., in 1998. This fungus was previously known only from the type specimen collected on *Picea pungens* in France and described in 1980 (Morelet 1980). Subsequent surveys by the Wisconsin Department of Agriculture, Trade and Consumer Protection indicated that this fungus occurred in 21 counties in Wisconsin on both Colorado blue spruce and white spruce (*Picea glauca* (Moench) Voss) (A. Mengistu, unpublished data). The fungus is consistently associated with plants exhibiting one or more symptoms including needle chlorosis and needle drop. Small, black perithecioid fruiting bodies develop on twigs during late May and early June, while needles are still present. Ascospores can be obtained from squashed ascomata collected in spring and early summer but are less common or absent from samples collected later in the year.

The monotypic genus *Setomelanomma* M. Morelet, based on *S. holmii*, was described initially from France on living twigs of *Picea pungens* and has not been reported since. The brief original description lacked illustrations and was published as a short communication in a regional journal (Morelet 1980). The holotype specimen was obtained and compared with the fungus from Wisconsin, U.S.A. Previously collected but misidentified specimens from Kansas, U.S.A., and Ontario, Canada, were located at the U.S. National Fungus Collections (BPI) and the National Mycological Herbarium of Canada (DAOM). After microscopic examination and comparision of molecular sequences, the fungus on spruce twigs in North America was determined to be *S. holmii*. This fungus is described in detail and illustrated below. The small subunit (SSU) nuclear ribosomal DNA (nrDNA) and the internal transcribed spacer (ITS) regions 1 and 2, including the 5.8S ribosomal DNA (rDNA), genes were sequenced to determine the phylogenetic affinities of this species.

Received 24 July 2002. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 5 December 2002.

A.Y. Rossman,[1] D.F. Farr, and L.A. Castlebury. USDA Agricultural Research Service, Systematic Botany and Mycology Laboratory, Room 304, B011A, 10300 Baltimore Boulevard, Beltsville, MD 20705, U.S.A.
R. Shoemaker. Agriculture Canada, Research Branch, Room 305, Saunders Building, Ottawa, ON K1A 0C6, Canada.
A. Mengistu. USDA Agricultural Research Service, Crop Genetics and Production Research Unit, Stoneville, MS 38776, U.S.A.

[1]Corresponding author (e-mail: arossman@nt.ars-grin.gov).

Materials and methods

For microscopic examination, material was rehydrated and

 DOI: 10.1139/B02-111

mounted in 3% KOH. Ascomata were sectioned at ca. 10 µm thick using a freezing microtome. Sections were mounted in lactic acid with cotton blue. Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with bright-field illumination. Photographs and measurements of microscopic features were taken using a Spot 2 digital camera (Diagnostic Instruments, Inc., Sterling Heights, Mich.) and ImagePro software (Media Cybernetics, Silver Spring, Md.). Single ascospores were suspended in sterile water and plated on 1.7% Difco corn meal agar (CMA) plus 0.2% dextrose and antibiotics (CMAD). Germinated ascospores were transferred to plates of 3.9% Difco potato dextrose agar (PDA), CMAD, and 1.5% water agar (WA) plus an alfalfa stem (*Medicago sativa* L.) for observation. Color names were determined using Rayner (1970). Specimens and cultures examined are listed following the description.

DNA extraction, purification, and amplification

DNA was extracted with the DNeasy Plant Mini kit (Qiagen Inc., Chatsworth, Calif.) according to the manufacturer's instructions using approximately 15 mg dried tissue from the following isolates of *S. holmii*: PC 99.4334 (extype culture) and AR 3666 (CBS 110217). Genomic DNA was also extracted from one strain of *Herpotrichia parasitica* R. Hartig (CBS 451.73). The SSU nrDNA was amplified from both isolates of *S. holmii* using the primer pairs NS1–NS8 and the ITS regions were amplified from PC 99.4334, CBS 110217, and CBS 451.73 with ITS5–ITS4 (White et al. 1990) in 50-µL reactions on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, Calif.) under the following reaction conditions: approximately 10 ng of genomic DNA, 200 mM each dNTP, 2.5 units AmpliTaq Gold (Applied Biosystems, Foster City, Calif.), 25 pmol of each respective primer and the supplied 10 × polymerase chain reaction (PCR) buffer with 15 mM $MgCl_2$. The thermal cycler program was as follows: 10 min at 95°C followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, with a final extension period of 10 min at 72°C. After amplification, the PCR products were purified with QIAquick columns (Qiagen Inc., Chatsworth, Calif.) according to the manufacturer's instructions. Amplified products were sequenced in each direction with the BigDye version 2.0 kit (Applied Biosystems, Foster City, Calif.) on an ABI 310 automated DNA sequencer. ITS products were sequenced using the PCR primers and the SSU products were sequenced with the PCR primers, as well as NS3, NS5, and NS7 in the forward direction and NS2, NS4, and NS6 in the opposite direction (White et al. 1990).

Sequence editing, alignment, and analysis

Raw sequences were edited using Sequencher version 4.05 for Windows (Gene Codes Corp., Ann Arbor, Mich.). A BLAST search of the GenBank database with the ITS and SSU sequences of *S. holmii* was performed to identify the most similar available sequences in GenBank (Altschul et al. 1997). Pairwise sequence differences across the ITS1, 5.8S rDNA, and ITS2 regions between *S. holmii* and similar fungi were calculated using PAUP* 4.0b10 (Swofford 1998).

Small subunit nrDNA sequences were aligned manually in GeneDoc version 2.6.001 (Nicholas et al. 1997). The alignment consisted of 40 taxa and 1068 positions of the 5′ end of the SSU nrDNA, with 129 parsimony informative characters. Small subunit nrDNA gene trees were inferred by neighbor joining using the Kimura two-parameter distance as implemented in PAUP* 4.0b10 (Swofford 1998) and by maximum parsimony using the heuristic search option with the random addition sequence (1000 replications) with the MULTREES setting in effect, unlimited MAXTREES and the branch swapping (tree bisection–reconnection) option of PAUP* 4.0b10 (Swofford 1998). All molecular characters were unordered and given equal weight during the analysis. Gaps were treated as missing data.

Relative support for branches was estimated with 1000 bootstrap replications (Felsenstein 1985) with MULTREES off, unlimited MAXTREES, and 10 random sequence additions for maximum parsimony bootstraps. Sequences generated in this study were deposited in GenBank as *Setomelanomma holmii*: PC 99.4334 SSU, AY161121; ITS, AF525675; CBS 110217 SSU, AF525677; ITS, AF525674; *Herpotrichia parasitica*: CBS 451.73 ITS, AF525676.

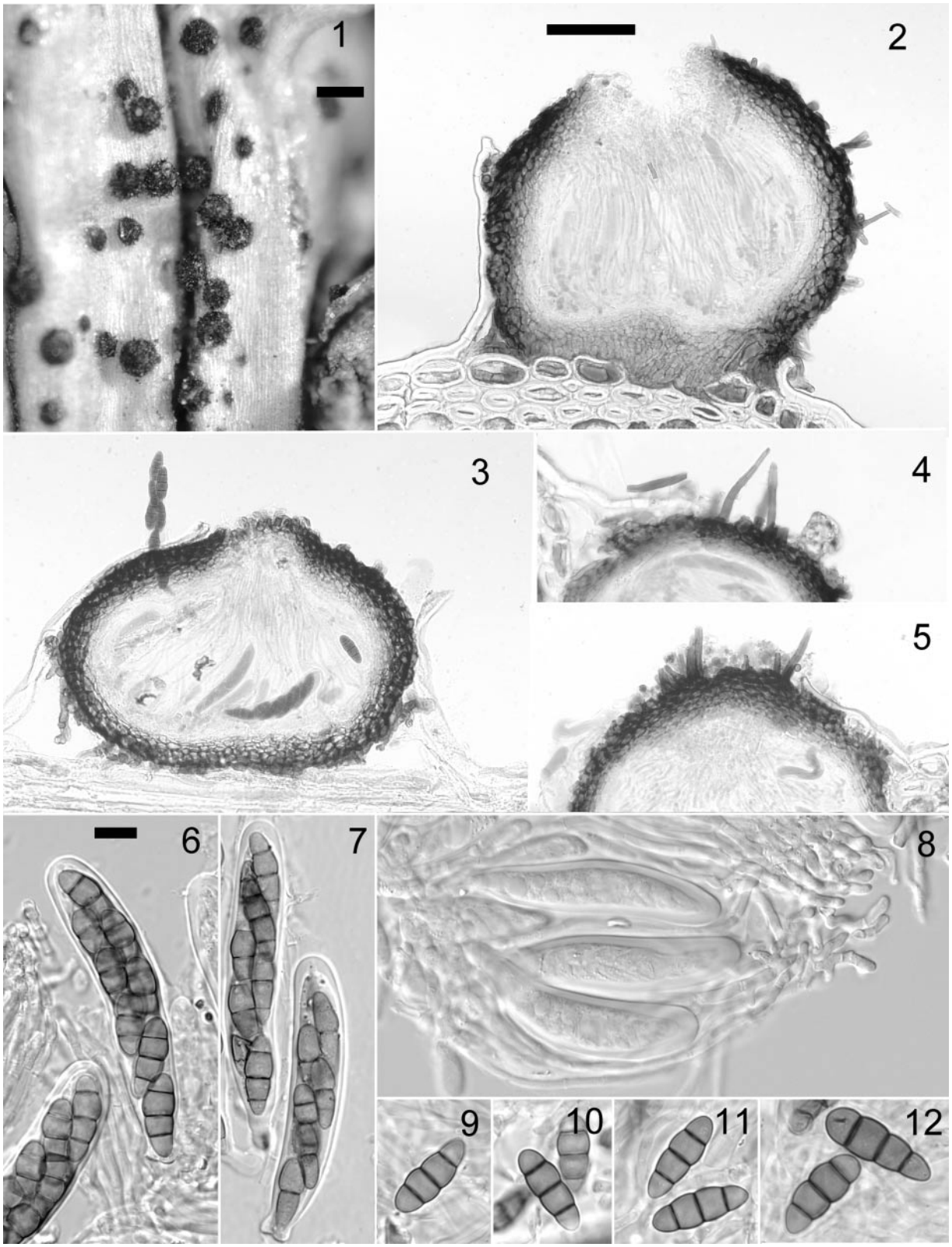
Taxonomy

Setomelanomma holmii M. Morelet, Bull. Sci. Nat. Archeol. Toulon, 36: 15, 1980. Figs. 1–12

Ascomata solitary, scattered, initially immersed, subcuticular, becoming erumpent, superficial through periderm of small twigs with needles still attached, perithecioid, 80–250 µm diameter, black, globose to subglobose, rarely collabent when dry, each with a nonrostrate, periphysate ostiolum, with scattered, sparse to abundant setae; setae short, brown, straight to flexuous, slightly tapered, blunt, 25–70 × 4–6 µm at base to 3 µm near apex. Ascomatal wall 15–25 µm thick, of four to seven layers of cells that are dark brown and thick walled toward outside, becoming thin walled and hyaline toward centrum, wall slightly thicker around nonpapillate ostiolum, of 5–10 layers of cells. Asci bitunicate, 65–85 × 12–15(–18) µm, broadly cylindrical with rounded apex, with short stalk, attached at base of ascomata, eight-spored. Interthecial elements arising from basal portion of ascomatal centrum, multicellular, 2–3 µm wide, septate, hyaline, anastomosing above asci. Ascospores broadly ellipsoidal, 16.4–22.5 × 5.5–7.4 µm (mean 20.0 × 6.3 µm, n = 53), pale to medium brown, three-septate, slightly constricted at primary, median septum, first and third septa secondary, cells about equal in length, widest at penultimate cell, apex broadly rounded, base rounded, surface smooth, gelatinous sheath not observed.

CULTURAL CHARACTERISTICS: On PDA, slow-growing, 7–8 mm diameter after 2 weeks, colony compact, raised in centre, with pale mouse-grey, short fluffy mycelium toward center, narrow margin, reverse sepia. On CMAD, slow-growing, 7–8 mm diameter after 2 weeks, colony compact, raised in centre with sparse, pale mouse-grey, fluffy mycelium only toward centre, flat, dark margin, reverse sepia. On WA plus alfalfa stem, slow-growing, 13–16 mm diameter after 24 days, flat around margin with hard clump in centre covered with dark aerial, fluffy hyphae, reverse dark mouse-grey to black, white toward margin. Ascomata did not develop in culture.

Figs. 1–12. *Setomelanomma holmii*. Fig. 1. Ascomata on needles of *Picea pungens* (BPI 841769). Figs. 2 and 3. Longitudinal section of ascomata (Fig. 2, DAOM 198318; Fig. 3, holotype). Figs. 4 and 5. Setae on ascomata (Fig. 4, BPI 841769; Fig. 5, holotype). Figs. 6 and 7. Mature asci (Fig. 6, holotype; Fig. 7, BPI 841769). Fig. 8. Interthecial elements among immature asci (Holotype). Figs. 9–12. Ascospores (DAOM 173151). Scale bars: Fig. 1 = 200 µm, Figs. 2–5 = 50 µm; Figs. 6–12 = 10 µm.



TYPE: France: Leuglay, on living twigs of *Picea pungens*, May 1978, C.N.R.F. 899 (holotype: CNRF; isotype: personal herbarium M. Morelet; isotype slide: UPS; ex-type culture PC 99.4334 ≡ AR 3804).

ADDITIONAL SPECIMENS EXAMINED: Canada: Ontario, Kingston, Potter's Nursery, on twigs of *Picea pungens* var. *glauca*, 20 May 1979, coll. D.M. Laidlaw, det. R.A. Shoemaker (DAOM 173151 as *Leptosphaeria praetermissa*); Toronto, 38 Pasadena Gardens, on dying twig of *Picea pungens*, 19 Aug 1975, coll. Bill Baker, det. R.A. Shoemaker (DAOM 150889 as *Leptosphaeria praetermissa*); Woodstock, Woodstock Tree Farm, on *Picea pungens*, 24 Sept. 1985, coll. Klenk 85–13, det. R.A. Shoemaker (DAOM 193569 as *Melanomma sparsum*); Markham, 21 Ridgedale Dr., on *Picea pungens*, 25 Mar 1988, coll. Wendy Gorniak, det. R.A. Shoemaker (DAOM 198318 as *Melanomma sparsum*). U.S.A: Kansas, Shawnee Co., on twigs of *Picea pungens,* 5 May 1959, coll. Herbert Bulk, (BPI 620251 as *Leptosphaeria* sp.); Wisconsin, on dying twigs of *Picea pungens*, July 2001, coll. A. Mengistu, cultured from ascospores by A. Rossman (AR 3666 ≡ CBS 110217) (BPI 841769); Montello, on *Picea* sp., 28 May 1930, coll. G.F. Massey, det. A.M.W. (DAOM 124092 as *Melanomma sparsum*).

Results

Setomelanomma holmii was originally described on *Picea pungens* from France by Morelet (1980). Several portions of the type specimen were examined and compared with the specimens from North America. Specimens from Canada (Ontario) and the U.S.A. (Kansas, Wisconsin) were determined to be the same as this species. The specimens from North America occur on *Picea pungens* (=*P. pungens* var. *glauca* Regel) as well as *Picea glauca* from as early as 1930. Additionally, the ex-type culture of *S. holmii* was examined and determined to be the same as a culture derived from ascospores of a Wisconsin specimen in growth rate and general appearance. ITS sequences for the two strains (AF525674, AF525675) differed only at three positions, all located in the ITS1 region.

Setomelanomma holmii is placed in the Phaeosphaeriaceae based on morphological characteristics. The relatively small, globose, perithecioid ascomata are immersed, becoming erumpent at maturity on 1- or 2-year old spruce twigs (Fig. 1). The ascomata each have a distinct, periphysate ostiolum and are composed of small, slightly thick-walled, black to hyaline, thin-walled, pseudoparenchymatous cells (Figs. 2 and 3). The upper portions of the ascomata have sparse to numerous, black, thick-walled setae that vary considerably in abundance among specimens (Figs. 4 and 5). Interthecial elements are present as cellular pseudoparaphyses that are branched above the asci and appear to be attached at both the apex and the base (Figs. 3 and 8). The asci are broadly cylindrical, bitunicate appearing fissitunicate (Figs. 6 and 7). The ascospores are pale to medium brown, transversely septate, slightly constricted at the septa (Figs. 9–12). Initially the ascospores have one, median septum with the two additional septa developing by maturity. No anamorph is known for *S. holmii* on natural substrata or in culture on agar alone or with alfalfa stems.

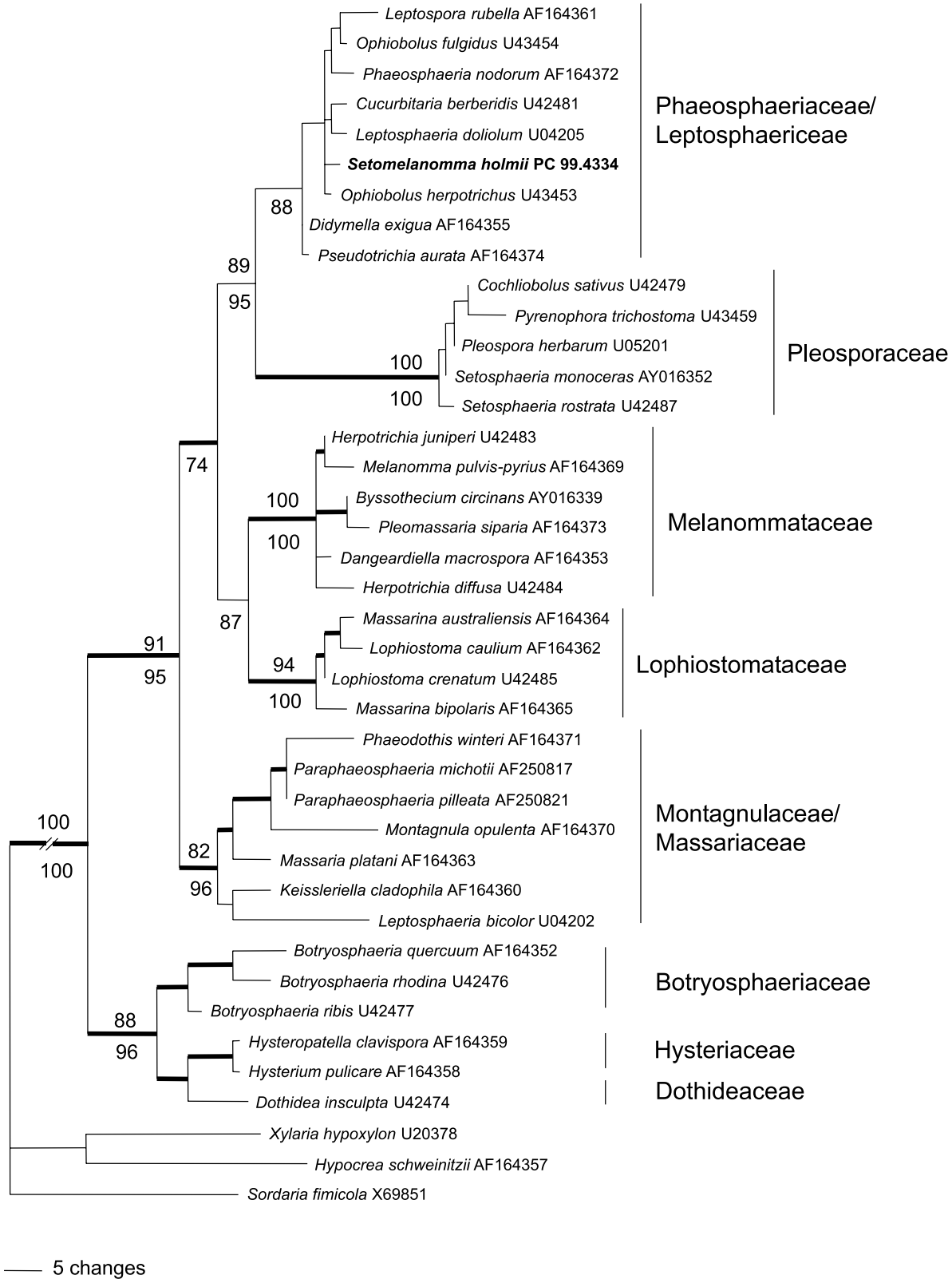
A BLAST search of GenBank using the ITS sequence of *S. holmii* (CBS 110217) returned sequences from *Phaeosphaeria juncina* CBS 594.86 (407 of 437 identities, 3 of 437 gaps) and *Phaeosphaeria culmorum* CBS 569.86 (408 of 436 identities, 6 of 436 gaps) as the best two matches available. This suggests a close relationship between *S. holmii* and species of *Phaeosphaeria*. Pairwise distances between *S. holmii* and *Phaeosphaeria juncina* and *Phaeosphaeria culmorum* were 11.1 and 13.6%, respectively. Maximum parsimony sequence analysis of the SSU nrDNA for representative members of the Phaeosphaeriaceae, Leptosphaeriaceae, and other loculoascomycetes resulted in 125 equally parsimonious trees with the following statistics: length = 377, CI = 0.674, RI = 0.834, RC = 0.562, and HI = 0.326. One arbitrarily chosen tree is shown in Fig. 13 with thickened lines indicating branches present in the strict consensus of all 125 trees.

Discussion

The type specimen of *S. holmii* from France and the specimens from North America are remarkably similar in morphological and cultural characteristics. Although known only from the type collection in Europe, this species was discovered under various names in herbaria in North America. The ITS sequence data also confirm the conspecificity of the North American fungus with that from France.

Considerable confusion has surrounded this fungus on spruce, now identified as *S. holmii*. Over the decades starting in 1930, specimens of the fungus have been deposited in BPI and DAOM as *Leptosphaeria* sp., *Leptosphaeria praetermissa* (P. Karst.) Sacc., and *Melanomma sparsum* Fuckel. Neither *Leptosphaeria praetermissa* nor *Melanomma sparsum* is the correct name or synonym for this fungus. Based on Huhndorf (1992) who examined the type specimen and redescribed the species, *Leptosphaeria praetermissa* belongs in *Leptosphaeria* Ces. & De Not., sensu stricto, and is restricted to species of *Rubus* L. (Rosaceae). Holm (1957) redescribed *Melanomma sparsum* based on two portions of the type specimen. In addition, several specimens at BPI were examined, namely BPI 622371 (Switzerland: Ragaz, "auf hartem Tannenholze, im Herbst", coll. Fuckel, Herb. Herbier Barbey-Boissier No. 608, Herbier Fuckel 1894, possible isotype) and BPI 622372 (U.S.A: Kansas, Rockport, on *Pinus* sp. – weathered boards, Feb. 1894, coll. E. Bartholomew, Ellis & Everhart Fungi Columbiani No. 3112). *Melanomma sparsum* occurs in well-rotten, decorticated wood of conifers, rather than on twigs; the ascomata are strongly flattened, cupulate when dry, and lack setae; the asci are numerous and broader than those of *S. holmii*. The fungus on spruce is morphological quite different from *M. sparsum*. *Setomelanomma holmii* was also considered to be related to *Nematostoma parasitica* (R. Hartig) M.E. Barr (≡*Herpotrichia parasitica*), a species that occurs on living needles of *Abies* spp. and is reported from Canada (Ontario), Denmark, and Switzerland (Barr 1997). The anamorph of *N. parasitica* was described as *Pyrenochaeta parasitica* by von Freyer and van der Aa (1975). Unlike *S. holmii*, *N. parasitica* has superficial ascomata, each surrounded by a sparse, superficial subiculum characteristic of the family Pseudoperisporiaceae in which this genus is placed. In addi-

Fig. 13. One of 125 equally most parsimonious trees resulting from analysis of 1068 base pairs of the 5′ small subunit nuclear rDNA of some loculoascomycetous fungi, including *Setomelanomma holmii*. Length = 377, CI = 0.674, RI = 0.834, and RC = 0.562. Values above and below the branches are parsimony and neighbor joining bootstrap supports, respectively, expressed as percentages. Thickened lines are branches that were present in the strict consensus tree.



tion the ascospores of *N. parasitica* are "asymmetric, with a strong tendency to be inequilateral or slightly curved" (Barr 1997). Pairwise base differences between ITS regions of *S. holmii* and *H. parasitica* (CBS 451.73, AF525676) were 23.9%.

The genus *Setomelanomma* based on *S. holmii* is similar to other genera of the Phaeosphaeriaceae in having broadly cylindrical, bitunicate asci, cellular interthecial elements, and pale brown, septate ascospores in which the primary septum is median. *Setomelanomma* can be distinguished from all other genera in the Phaeosphaeriaceae as defined by Barr (1992) and Eriksson et al. (2002) by the brown, three-septate ascospores, setose ascomata, and its occurrence on conifers. In the key to genera of the Phaeosphaeriaceae in North America (Barr 1992), *Setomelanomma* keys closest to *Kalmusia* and *Phaeosphaeria*. Species of *Kalmusia* have ascomata that remain immersed at maturity and have a well-developed apical papillae, characteristics unlike *Setomelanomma*. In a study analyzing ITS sequences, the asexual state of the type species *Kalmusia coniothyrium* (Fuckel) Huhndorf (as *Coniothyrium fuckelii* Sacc., = *Coniothyrium sporulosum* (W. Gams & Domsch) Aa) was grouped with species distant from *Phaeosphaeria nodorum* and other genera in the Phaeosphaeriaceae (Muthumeenakshi et al. 2001). Pairwise base differences between the ITS regions of *S. holmii* (CBS 110217, AF525674) and *C. fuckelii* (CBS 132.26, AJ293813) were 28.5%.

Except for the presence of setae and its occurrence on conifers, *S. holmii* is morphologically similar to *Phaeosphaeria*. The genus *Phaeosphaeria* is centered around the type species, *P. oryzae* Miyake, and *P. nodorum* (E. Müll.) Hedjaroude, both species causing diseases of grain crops (Hedjaroude 1968; Leuchtmann 1984). Most species of *Phaeosphaeria* are known from monocotyledonous hosts, primarily grasses (Poaceae) and sedges (Cyperaceae), although some species occur on dicotyledonous hosts especially the Caryophyllaceae and pteridophytes, specifically *Lycopodium* and *Selaginella* (Holm 1957, Leuchtmann 1984; Shoemaker and Babcock 1989). While some species of *Phaeosphaeria* have coelomycetous anamorphs, *S. holmii* apparently lacks an anamorphic state.

Analysis of SSU nrDNA sequences of *Setomelanomma* and genera in the Pleosporales confirms the placement of *S. holmii* in the clade containing representatives of the Phaeosphaeriaceae and Leptosphaeriaceae (Fig. 13). *Setomelanomma* groups with *Cucurbitaria*, *Didymella*, *Leptosphaeria*, *Leptospora*, *Ophiobolus*, *Ophiosphaerella*, *Phaeosphaeria*, and *Pseudotrichia*, all placed in the Phaeosphaeriaceae or the closely related Leptosphaeriaceae. Although these two families are distinguished by Kirk et al. (2001) and Eriksson et al. (2002) based on the presence–absence of scleroplectenchymatous cells around the ostiole, they could not be resolved using the taxa for which SSU nrDNA sequences were available. The high divergence among ITS sequences, including insertion and deletion events, among members of the Leptosphaeriaceae and Phaeosphaericaceae precludes the meaningful alignment of sequences for taxa across these two families. Sequences of additional genes will be required to more definitively sort out relationships among these fungi. Other families of Loculoascomycetes represented in Fig. 13 at bootstrap levels above 80% include the genera in the Pleosporaceae, Montagnulaceae, Melanommataceae, and Lophiostomataceae in the Pleosporales and a group containing members of the Botryosphaericeae, Hysteriaceae, and Dothideaceae. It should be noted that *Setomelanomma* in the Phaeosphaeriaceae is quite unlike the genus *Melanomma* represented by the type species, *M. pulvis-pyrius*, in the Melanommataceae.

Acknowledgements

The authors acknowledge Ove Eriksson for bringing the name *Setomelanomma holmii* to our attention and providing a slide of the type specimen. In addition, the original author of this taxon, Michel Morelet, as well as Jean Pinon provided portions of the type specimen for our examination. Finally, Jean Mouchacca and Marie-France Roquebert sent the ex-type culture. Insight into the possible the placement of this fungus was gained from discussions with Margaret Barr Bigelow. For all of this support from the international community of mycologists, we are extremely grateful. We also acknowledge Aimee Sheer for her skillful technical assistance.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J, Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**: 3389–3402.

Barr, M.E. 1992. Additions to and notes on the Phaeosphaeriaceae (Pleosporales, Loculoascomycetes). Mycotaxon, **43**: 371–400.

Barr, M.E. 1997. Notes on some dimeriaceous fungi. Mycotaxon, **64**: 149–171.

Eriksson, O.E., Baral., H.O., Currah, R.S., Hansen, K., Kurtzman, C.P., Rambold, G., and Laessøe, T. 2002. Outline of Ascomycota — 2002. Myconet. Available from http://www.umu.se/myconet/Myconet.html.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **6**: 227–242.

Hedjaroude, G.A. 1968. Études taxonomiques sur les *Phaeosphaeria* Miyake et leurs formes voisines (Ascomycetes). Sydowia, **22**: 57–107.

Holm, L. 1957. Études taxonomiques sur les Pléosporacées. Symb. Bot. Ups. **14**: 1–188.

Huhndorf, S.M. 1992. Systematics of *Leptosphaeria* species found on the Rosaceae. Illinois Natural History Survey, Champaign, Ill. Biol. Notes No. 34. pp. 480–533.

Kirk, P.M., Cannon, P.F., David, J.C., and Stalpers, J.A. 2001. Ainsworth & Bisby's dictionary of the fungi. CAB International, Wallingford, England.

Leuchtmann, A. 1984. Über *Phaeosphaeria* Miyake und andere bitunicate Ascomyceten mit mehrfach querseptierten Ascosporen. Sydowia, **37**: 75–194.

Morelet, M. 1980. Sur quatre Dothideales. Bull. Soc. Sci. Nat. Archeol. Toulon, **36**: 14–15.

Muthumeenakshi, S., Goldstein, A.L., Stewart, A., and Whipps, J.M. 2001. Molecular studies on intraspecific diversity and phylogenetic position of *Coniothyrium minitans*. Mycol. Res. **105**: 1065–1074.

Nicholas, K.B., Nicholas, H.B., Jr., and Deerfield, D.W., II. 1997. GeneDoc: analysis and visualization of genetic variation. EMBNEW News, **4**: 14.

Rayner, R.W. 1970. A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey, England.

Shoemaker, R.A., and Babcock, C.E. 1989. *Phaeosphaeria*. Can. J. Bot. **67**: 1500–1599.

Swofford, D.L. 1998. PAUL*4.0. Phylogenetic analysis using parsimony. Sinauer Associates, Sunderland, Mass.

von Freyer, K., and van der Aa, H.A. 1975. Über *Pyrenochaeta parasitica* spec. nov., die Nebenfruchtform von *Herpotrichia parasitica* (Hartig) E. Rostrup (= *Trichosphaeria parasitica* Hartig). Eur. J. For. Pathol. **5**: 177–182.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, S.S. Sninsky, and T.J. White. Academic Press, San Diego, Calif. pp. 315–322.